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THE USE OF ULTRAVIOLET RADIATION AS A NON-THERMAL TREATMENT FOR THE INACTIVATION OF ALICYCLOBACILLUS ACIDOTERRESTRIS SPORES IN WATER, WASH WATER FROM A FRUIT PROCESSING PLANT AND GRAPE JUICE CONCENTRATE

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Abstract

Alicyclobacillus acidoterrestris is a non-pathogenic, spore-forming bacterium that can survive the commercial pasteurisation processes commonly used during fruit juice production. Surviving bacterial endospores germinate, grow and cause spoilage of high acid food products. Fruit juices can be treated using ultraviolet light (UV-C) with a wavelength of 254 nm, which has a germicidal effect against micro-organisms. In this study, *A. acidoterrestris* was inoculated into water, used wash water from a fruit processing plant and grape juice concentrate. Ultraviolet dosage levels (J L⁻¹) of 0, 61, 122, 183, 244, 305 and 367 J L⁻¹ were applied using a novel UV-C turbulent flow system. The UV treatment method was shown to reliably achieve in excess of a 4 log₁₀ reduction (99.99%) per 0.5 kJ L⁻¹ of UV-C dosage in all the liquids inoculated with *A. acidoterrestris*. The applied novel UV technology could serve as an alternative to thermal treatments of fruit juices for the inactivation of *Alicyclobacillus* spores as well as in the treatment of contaminated wash water used in fruit processing.

Keywords: fruit juice; *Alicyclobacillus acidoterrestris*; spoilage; non-thermal processing; ultraviolet radiation

1. INTRODUCTION

Alicyclobacillus acidoterrestris is a Gram-positive, thermo-acidophilic, non-pathogenic, spore-forming bacterium that has been isolated and identified in spoiled commercial pasteurised fruit juices (Wisotskey et al., 1992; Walls & Chuyate, 1998; Silva & Gibbs, 2000; Fredericks et al., 2011). This bacterium was initially isolated by Hippchen et al. (1981) from a variety of different soils such as garden soil, oak wood soil, woodland soil and the soil found in moor lands. *Alicyclobacillus acidoterrestris* has subsequently been found in a range of habitats and substrates including organic composting, manure, crop fields, orchards, heat-processed foods such as fruit juice concentrates, and the fruit juice concentrate processing environment (Deinhard et al., 1987; Yamazaki et al., 1996; Pettipher et al., 1997; Albuquerque et al., 2000; Walls

and Chuyate, 2000; Goto et al., 2002; Matsubara et al., 2002; Groenewald et al., 2008 and 2009).

The threat that *A. acidoterrestris* poses to the fruit juice industry is the ability of its spores to survive thermal pasteurisation as well as the hot-fill and hold pasteurisation processes used during fruit processing and fruit juice production (Splittstoesser et al., 1994; Eiroa et al., 1999; Orr & Beuchat, 2000). This heat resistance was observed by Splittstoesser et al. (1994) who reported D-values for *A. acidoterrestris* spores of 23 min at 90°C and 2.4 to 2.8 min at 95°C, suggesting that these spores survive the juice pasteurisation process of 88°C to 96°C for 30 s. to 2 min. In fact, pasteurisation serves as a heat treatment that stimulates germination of the spores. The resulting growth of *A. acidoterrestris* at the low pH (3 - 3.5) typically found in fruit juice may lead to spoilage (Splittstoesser et al., 1998; Eiroa et al., 1999; Gouws et al., 2005).

To date, spoilage caused by *A. acidoterrestris* has been reported in apple, pear, orange, peach, mango and white grape juice as well as in fruit juice blends, fruit juice containing drinks and tomato products such as tomato juice and canned tomatoes (Borlinghaus & Engel, 1997; Chang & Kang, 2004). Spoilage caused by this bacterium is difficult to detect visually. The spoiled juice appears normal, or it might have light sediment with no visible gas formation. Often, the only evidence of spoilage is apparent as a medicinal/phenolic off-flavour (Walls & Chuyate, 1998; Jensen, 1999). The chemicals responsible for this off-odour were identified as guaiacol (2-methoxyphenol) and other halophenols such as 2,6-dichlorophenol (2,6- DCP) and 2,6-dibromophenol (2,6-DBP). Guaiacol can be detected by a smell in fruit juices at 2 ppb and was detected in orange and apple juices in the presence of around 5 log CFU mL⁻¹ of *A. acidoterrestris* cells by Gocmen et al. (2005).

Fruit juice contamination results from unwashed or poorly washed raw fruit that is processed as well as from contaminated water used during the production of fruit juices (Pontius et al., 1998; Orr & Beuchat, 2000; McIntyre et al., 1995; Groenewald et al., 2009). Due to their thermo-acidophilic properties and their occurrence in several spoiled pasteurised products, Silva et al. (1999) recommend *A. acidoterrestris* spores as the target microbe for the pasteurisation of high acidic food products. Subsequently, the fruit juice industry acknowledges *A. acidoterrestris* as an important target microorganism that must be managed by an effective quality control program during the production of fruit juices and fruit juice concentrates.

Ultraviolet (UV) light is one of a number of non-thermal technologies currently being used in food processing. Other technologies include pulsed electric fields, high-pressure processing and ultrasound. These technologies can deliver food products that neither suffer from spoilage nor contain pathogenic micro-organisms and enzymes that may decrease the nutritional and sensory characteristics of foods (Butz & Tauscher, 2002; Fredericks et al., 2011).

UV wavelengths of between 220 and 300 nm are considered germicidal against micro-organisms such as bacteria, viruses, protozoa, fungi and algae (Sizer & Balasubramaniam, 1999; Bintsis et al., 2000). The highest germicidal effect is obtained between 250 and 270 nm; this decreases as the wavelength is increased. Above 300 nm, the germicidal effect of UV light is annulled. Therefore, a wavelength of 254 nm is used for disinfection of surfaces, water and some food products (Guerrero-Beltran & Barbosa-Canovas, 2004; Lin et al., 2012).

Liquids such as water and fruit juices have been successfully treated with UV light to reduce bacterial counts (Guerrero-Beltran & Barbosa-Canovas, 2004; Keyser et al., 2008). The efficacy of the microbial reduction in fruit juices by UV-C light at 254 nm depends on a number of factors. These include the organisms (including different strains) present in the liquid, the contamination level, the percentage UV transmittance of the liquid (opaqueness) and the percentage of suspended particles in the liquid. It is known that the penetration depth of UV-C light through the surface of liquids is very short, with the exception of clear water (Shama, 1999). The penetration of UV light into juices is about 1mm for absorption of 90% of the light (Sizer & Balasubramaniam, 1999). Greater amounts of soluble and insoluble solids lower the intensity of penetration of the UV-C light (Shama, 1999; Bintsis et al., 2000). For these reasons, a turbulent flow during liquid food processing is not only recommended, but it is a legal requirement by the USA Food and Drug Administration (US FDA, 2001; Keyser et al., 2008; Simmons et al., 2012). The objective of this study was to determine the reduction of *A. acidoterrestris* spores inoculated in water, fruit concentrate factory wash water and 80 °Brix grape juice concentrate using a novel UV treatment system.

2. MATERIALS AND METHODS

2.1 Novel pilot-scale UV system

The UV reactor system used in this study was designed and manufactured by SurePure, Milnerton, South Africa. The UV reactor (Fig. 1) consists of NW100 stainless steel inlet and outlet chambers with a stainless steel corrugated spiral tube between the chambers. Inside the spiral tube is a low pressure mercury UV lamp (30 UV-C Watt, 90% 254 nm and 90% emittance) which is protected by a quartz sleeve. The liquid flows between the corrugated spiral tube and the quartz sleeve. The tangential inlet of the reactor creates a high velocity and turbulence in the inlet chamber which helps to prevent clumping of micro-organisms and assists in the efficiency of UV radiation by increasing the exposure of the liquid to the light. From the inlet chamber the liquid is forced into the actual reactor through the space between the quartz sleeve and the corrugated spiral tubing. The corrugation of the tubing creates very high turbulence which is then carried along the spiral over the length of the reactor chamber.

At flow rates (Fr) above $2\,800\text{ L h}^{-1}$ the Reynolds value is calculated to be more than 4 000, indicating turbulent flow patterns. The UV reactor operates at a flow rate capacity of between $3\,800$ and $4\,200\text{ L h}^{-1}$.

The time needed for a UV treatment depends on the quantity of product to be treated and the flow rate of the product feed. The design of the pilot-scale batch system (Fig. 1) that was used consists of only one UV lamp. This unit was used for the treatment of 20 L batches of liquid which were circulated at a flow rate of $4\,000\text{ L h}^{-1}$. The time for the liquid to pass through the system once was 18 s, thereby delivering a UV-C dose of 22.97 J L^{-1} to the liquid being treated after one passage. The contact time or retention time was determined theoretically, assuming that the system was in steady state operation with uniform product and product flow and that the liquid was non-expandable and non-volatile.

2.2 Cleaning of the units

The pilot-scale unit was cleaned after every treatment using standard 'Cleaning in Place' (CIP) processes. The equipment was rinsed with warm water ($50\text{ }^{\circ}\text{C}$) for 10 min after which a 1.0% alkaline solution was circulated for 30 min at $75\text{ }^{\circ}\text{C}$. This was followed by a warm water rinse at $50\text{ }^{\circ}\text{C}$ for 5 min. Finally, a 0.5% Perasan solution (Divosan System, Johnson Diversey, South Africa) was circulated through the unit for 10 min before it was finally rinsed with cold water.

2.3 Dosage measurement

As UV light was used initially to disinfect surfaces, the irradiance is generally expressed as watts per square centimetre (W cm^{-2}) whilst the radiant exposure (dosage) is expressed as watts per second per square centimetre (W s cm^{-2}) or joules per square centimetre (J cm^{-2}) (Matak et al., 2005). UV dosage (D) is therefore determined as time (T) multiplied by irradiance (I). As the UV-C energy penetrates into the medium, therefore working with volume rather than area, Keyser et al. (2007) proposed an alternative method to characterize UV as dosage per volume of liquid. For liquids, the UV dosage is expressed as J L^{-1} . A comparison between UV-C dosage as J L^{-1} and W cm^{-2} was therefore determined (Table 1) by calculating the dose per area as well as the dose per volume, together with time of UV-C exposure.

2.3.1 UV dosage per area

The length of the quartz sleeve used was 0.860 m with an outer surface area (A_s) of 661.93 cm^2 . The area between the quartz sleeve and the corrugated spiral tubing is termed the annulus and the volume thereof was determined as being 0.675 L or 0.00068 m^3 . The effective area (A_s) of UV-C was at a distance of 5 mm as the lamp was 5 mm away from the outer surface of the sleeve.

According to the manufacturers, the energy transmission rate (total UV-C output) to the constant surface of the quartz sleeve ($A_s = 661.93 \text{ cm}^2$) from the UV lamp is 25.5 W (watts) UV-C. Disregarding the volume of the annulus and the type of product in the annulus, the following calculations were based on the effective A_s of the quartz sleeve alone.

The intensity (I) per reactor was calculated as follows:

$$\text{Intensity (I)} = \text{Total UV-C output per unit (W)} / \text{Area (cm}^2\text{)} = 25.5 \text{ W} / 661.93 \text{ cm}^2 = 0.039 \text{ W cm}^{-2} = 38.5 \text{ mW cm}^{-2}$$

The retention time (T) of the product per reactor was calculated as follows:

$$\text{Retention time (T)} = \text{Volume of the reactor (l)} / \text{Flow rate L h}^{-1} = 0.675 \text{ L} / 4000 \text{ L h}^{-1} = 0.675 \text{ L} / 1111 \text{ L s}^{-1} = 0.608 \text{ s}$$

Thus, at a flow rate (Fr) of 4 000 L h⁻¹, the product retention time (T) was 0.608 s per reactor; therefore the UV dosage (D) per surface area for one reactor with continuous flow was calculated as follows:

$$\text{Dosage} = \text{Intensity (I)} \times \text{Time (T)} = 38.50 \text{ mW cm}^{-2} \times 0.608 \text{ s} = 23.408 \text{ mW s cm}^{-2} = 23.408 \text{ mJ cm}^{-2}$$

2.3.2 UV dosage per volume

At a flow rate (Fr) of 4 000 L h⁻¹, the product retention time (T) was 0.608 s per reactor (as presented in paragraph 2.3.1), therefore the UV dosage per L of liquid treated for one reactor with continuous flow was calculated as follows:

$$\text{Dosage} = \text{Total UV-C output per unit (W)} / \text{Flow rate (L s}^{-1}\text{)} = 25.50 \text{ W} / 1.11 \text{ L s}^{-1} = 25.50 \text{ J.s}^{-1} / 1.11 \text{ L s}^{-1} = 22.972 \text{ J L}^{-1}$$

2.4 Growth of *Alicyclobacillus acidoterrestris*

Alicyclobacillus acidoterrestris K47 (Witthuhn et al., 2007), a strain isolated from spoiled grape juice, was grown in 2 L yeast starch glucose (YSG) broth (Matsubara et al., 2002), adjusted with tartaric acid (1N) (Saarchem, Krugersdorp, South Africa) to a final pH of 4 and incubated at 45 °C for 5 days.

This culture was then heat treated at 80°C for 10 min to promote the germination of any *Alicyclobacillus* spores and to eliminate vegetative cells (Walls & Chuyate, 2000) before being used as an inoculum. *Alicyclobacillus acidoterrestris* spores were inoculated by the addition of the whole pellet into either water, used wash water from a fruit processing plant or 80 °Brix grape juice concentrate. A final concentration of approximately $5 \times 10^5 \text{ CFU mL}^{-1}$, as determined by sampling at time 0, was obtained.

2.5 UV-C processing of used wash water and water

Used wash water was obtained from a Hazard Analysis Critical Control Point (HACCP) accredited fruit processing facility in the Western Cape region of South Africa and kept at 22 °C. The wash water, containing foliage and dust, had been used to wash off fruit debris and dust before processing and had been recycled several times. The wash water and the tap water were inoculated with around 5 log cfu.mL⁻¹ *A. acidoterrestris* K47 and processed in a similar way as the grape fruit concentrate, except that these liquids were processed at 22 °C. All the UV-C treatments were done in quadruplicate.

2.5.1 UV-C processing of 80 °Brix grape juice concentrate

Grape juice 80 °Brix concentrate had been obtained from a concentrate manufacturer in the Western Cape, South Africa and kept at 4 - 8 °C. A sample volume of 20 L was inoculated with *A. acidoterrestris* K47 as previously described and placed into the holding tank of the pilot UV treatment unit. To achieve a flow rate of 4 000 L h⁻¹ in the unit, a speed controlled sanitary Prolac centrifugal pump (Inoxpa, Brackenfell, South Africa) was used. The concentrate was treated at 4 - 8 °C and, due to the short contact time, no heat transfer from the lamps to the concentrate was recorded after processing. Samples were subjected to UV dosages of 0, 61, 122, 183, 244, 305 and 367 J L⁻¹. After each dosage, a 50 mL sample was taken aseptically using an in-line sampler. The concentrate was extracted from the flow stream without halting the process in order to avoid excessive UV-C exposure of the grape juice concentrate. Microbiological analyses were performed on each 50 mL sample within 24 h. All the UV-C treatments were done in quadruplicate.

2.6 pH determinations

Suspended particles in the wash water were allowed to settle and the pH was measured using a HI 221 pH meter (Hanna Instruments, Bedfordshire, United Kingdom).

2.7 Microbiological analysis

A 100 µL sample of grape juice concentrate, tap water or wash water was aseptically transferred to 900 µL sterile distilled water and mixed thoroughly.

Serial dilutions of the samples were then prepared (10⁻¹-10⁻⁶) and 100 µL of each of the different sample dilutions was plated in triplicate onto YSG agar. Tartaric acid (1N) (Saarchem) was used to adjust the YSG agar to a final pH of 4. Plates were

incubated aerobically at 45 °C and examined for growth after 96 h. The results obtained were expressed as colony forming units per milliliter (cfu mL⁻¹).

2.8 Statistical analyses

All the statistical analyses were performed using Statistica™ 7.1 (StatSoft, Inc., 2006). A two-way cross classification of the log cfu.mL⁻¹ on UV-C dosage and treated medium was carried out. Since this interaction was highly significant ($F_{12,63} = 5.31$) with a P-value of 0.000004, the interactions between the treated media (water, wash water and grape juice concentrate) and UV-C dosage were investigated. As the residuals were not normally distributed, a Bootstrap multiple comparison was performed on the interactions.

3. RESULTS AND DISCUSSION

UV radiation was successfully applied to reduce *A. acidoterrestris* spores inoculated into tap water, used wash water from a fruit concentrate manufacturing facility and 80 °Brix grape juice concentrate (Table 1). In water inoculated with *A. acidoterrestris* spores, a 5.3 log₁₀ reduction of the alicyclobacilli was achieved after a UV dosage of only 305 J L⁻¹, resulting in no viable spores (Fig. 2). The UV treatment method was shown to be capable of reliably achieving in excess of a 4 log₁₀ reduction (99.99%) after 500 J L⁻¹ of applied UV-C dosage in *A. acidoterrestris* inoculated in used fruit juice concentrate factory wash water (Fig. 2).

Alicyclobacillus acidoterrestris had previously been isolated from wash water in a factory that processes fruit by Groenewald et al. (2008). Wash water can act as a potential reservoir of *A. acidoterrestris*, resulting in the contamination of the fruit concentrate or juice product. Wash water is used during the production of fruit concentrates to wash the fruit and to remove dust, soil and any foreign objects from the fruit immediately prior to pulping. This water is conserved by recycling it during the manufacturing process; thus fruit can potentially be re-inoculated with *A. acidoterrestris* spores. Wash water can be subjected to UV treatment to decrease the contamination of fruit by *A. acidoterrestris* during processing. In the current study the wash water had a pH of 3.98.

Figure 2 represents the log reduction of *A. acidoterrestris* spores in 80 °Brix grape juice concentrate after UV treatment. It can be observed that a total inactivation of spores was obtained after 367.2 J L⁻¹ had been applied. This equates to around a 4.61 log₁₀ reduction in spores. The grape juice concentrate used had a pH of 2.8.

It was a clear liquid without any suspended solids which made it easier for the UV light to penetrate than if an opaque liquid had been used. Koutchma et al. (2004) posit that the factor that consistently affects the efficacy of UV light inactivation in juice is absorbance, while factors unique to juice such as °Brix and pH do not exhibit a

profound effect on the efficacy of the treatment. Although grape juice concentrate itself is not susceptible to spoilage due to its high sugar content (Chang & Kang, 2004), its contamination with *A. acidoterrestris* spores can lead to spoilage when the concentrate is diluted to single strength fruit juice and *A. acidoterrestris* spores find a favourable environment for growth. It has therefore been suggested that *A. acidoterrestris* becomes the target spoilage organism for effective pasteurisation and that the processes to achieve this must be designed to eliminate the spores (Silva & Gibbs, 2001). However, the high thermal resistance of these spores would necessitate pasteurisation at elevated temperatures, resulting in unacceptable changes in the organoleptic and nutritional characteristics of the treated fruit concentrate or juice.

The UV inactivation curves for *A. acidoterrestris* showed mainly linear regression with only a slight tailing effect in all three treated liquids (Fig. 2). The sigmoidal shape of the curve with a shoulder and tailing is described as typical for UV light inactivation of micro-organisms (Koutchma et al., 2004; Hoyer, 1998). The shoulder is attributed to the requirement for more than one UV light hit to kill a micro-organism. Tailing has been attributed to either variability in the UV light sensitivity of the targeted population, including variability of UV resistance genes turned on, or to non-uniform processing conditions due to laminar type of flow and shading effects owing to insufficient exposure to UV light in solutions of lower transmittance (Hoyer, 1998). In this study the linear regression observed might have been as a result of a single strain of *A. acidoterrestris* being used. This also suggests that the novel UV treatment system reached sufficient turbulent flow inside the reactor to ensure an even UV exposure. It is important to note that the current USA FDA regulations on the use of UV light for fresh juice stipulate the use of a turbulent flow system (US FDA, 2001).

A bootstrap multiple comparison between UV-C dosage and media (tap water, wash water and grape juice concentrate) found significant differences between applied UV-C dosages of 244 and 305 J L⁻¹. At an applied UV-C dosage of 244 J L⁻¹ on wash water, the surviving spores (1.54 log CFU mL⁻¹) were significantly higher than for a similar treatment on tap water (0.81 log CFU mL⁻¹) and on 80 °Brix grape juice concentrate (1.02 log CFU mL⁻¹), with $P = 0.0035$. At an applied UV-C dosage of 305 J L⁻¹, the surviving spores (log CFU mL⁻¹) in tap water were significantly lower (0.01) than for a similar treatment on wash water (0.70) and on 80 °Brix grape juice concentrate (0.64) (Table 1). The greater UV-C absorptivity of the used wash water and the grape juice concentrate was due to the presence of suspended matter and soluble solids respectively in the liquids.

Optimisation of the parameters is essential to ensure the maximum reduction of the microbial load of different fruit juices and concentrates without affecting the taste of the product. These parameters include the magnitude of turbidity, UV light transmittance through the media in the reactor, flow pattern and flow rate. Based on the results obtained in this study, it can be concluded that the use of the novel UV

treatment system is a promising way to control contamination of juice concentrates by species of Alicyclobacillus. However, additional research needs to be conducted to further evaluate the effect of UV on the organoleptic and nutritional characteristics of fruit juices. Moreover, further investigation is required into the effect of UV on the enzymes which can cause clarification or browning of the juice concentrate and the subsequent single strength juice produced from the treated concentrate.

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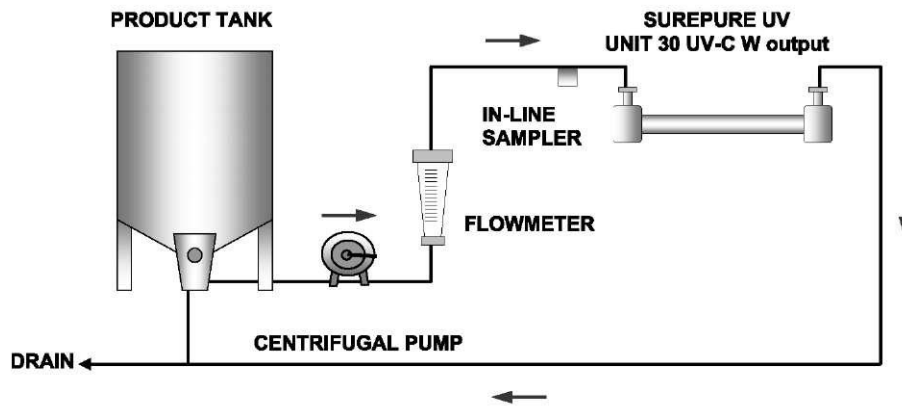


Figure 1: Schematic representation of the novel pilot-scale UV treatment system containing one UV-C lamp

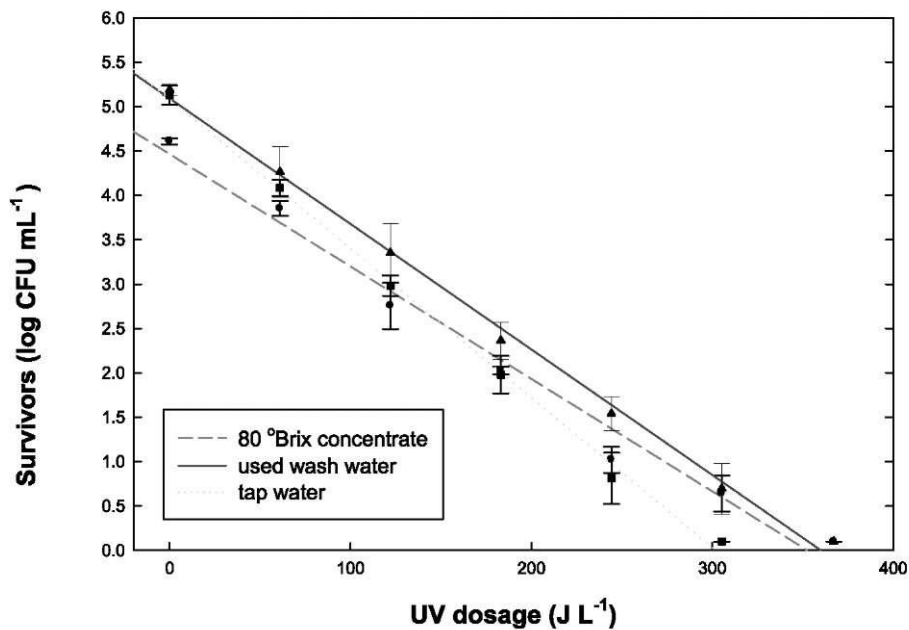


Figure 2 The log₁₀ reduction of *A. acidoterrestris* K47 spores at a starting concentration of approximately 5×10^5 cfu.ml⁻¹ in 80 °Brix grape juice concentrate, used fruit concentrate factory wash water and tap water. (Each data point represents quadruplicate values. The standard deviation was used as the error-bar)

Table 1: The log₁₀ microbial reduction of *A. acidoterrestris* (CFU mL⁻¹) (average value calculated from 4 repetitions) at a starting concentration of around 5×10^5 CFU mL⁻¹ in inoculated tap water, used wash water from a fruit processing plant and 80 °Brix grape juice concentrate after UV (J L⁻¹) treatment

medium

LogTM *A. acidoterrestris* reduction

	Applied UV dosages						
	0	61	122	183	244	305	367
Inoculated tap water	0.00	1.04 (0.89-1.28)	2.15 (1.96-2.31)	3.15 (2.96-3.29)	4.32 (4.06-4.29)	5.13 (4.99-5.23)	5.13 (4.99-5.23)
Inoculated used wash water	0.00	0.925 (0.43-1.13)	1.84 (1.43-2.21)	2.83 (2.59-3.03)	3.65 (3.49-3.95)	4.49 (4.29-4.91)	5.19 (5.11-5.27)
Inoculated 80 °Brix grape juice concentrate	0.00	0.76 (0.60-0.85)	1.85 (1.51-2.14)	2.59 (2.55-2.66)	3.59 (3.43-3.71)	3.97 (3.73-4.24)	4.61 (4.56-4.64)

The values given are averages (n = 4); values in parentheses are the minimum and maximum values of four samples.

Table 2: The log₁₀ microbial counts of *A. acidoterrestris* (CFU mL⁻¹) (average value calculated from 4 repetitions) in inoculated tap water, used wash water from a fruit processing plant and 80 °Brix grape juice concentrate after UV (J L⁻¹) treatment

Treated medium	Log ₁₀ <i>A. acidoterrestris</i>						
	UV dosages						
	0	61	122	183	244	305	367
Inoculated tap water	5.13	4.09	2.98	1.98	0.81	0.01	0.01
Inoculated used wash water	5.19	4.27	3.35	2.36	1.54	0.70	0.01
Inoculated 80 °Brix grape juice concentrate	4.61	3.85	2.76	2.03	1.02	0.64	0.01